"An Experimental Investigation of the *Rôle* of the Blood Fluids in connection with Phagocytosis." By A. E. WRIGHT, M.D., late Professor of Pathology, Army Medical School, Netley, Pathologist to St. Mary's Hospital, W., and STEWART R. DOUGLAS, M.R.C.S., Captain, Indian Medical Service. Communicated by Sir JOHN BURDON SANDERSON, Bart., F.R.S. Received September 1, 1903.

It is still a matter of uncertainty whether the blood fluids perform any *rôle* in connection with phagocytosis.

Certain facts suggest that the *rôle* of the blood fluids, if it comes into consideration at all, is very subordinate. The facts we have in view are, on the one hand, the facts brought forward by Metchnikoff to show that bacteria may be ingested in the living condition, and on the other hand those, brought forward by one of us in conjunction with Captain F. Windsor, I.M.S.,* which show that the human serum exerts absolutely no bactericidal action on the staphylococcus pyogenes, the micrococcus melitensis and the plague bacillus.

These facts are, however, not conclusive. They are not inconsistent with the idea that the blood fluids, apart from actually killing the particular pathogenic bacteria here in question, may in some way co-operate in their destruction.

What are required for the resolution of the problem are experiments in which the phagocytes are tested apart from the blood fluids.

The experimental methods which we now pass on to describe enable these crucial experiments to be made.

Methods of Experimentation.

We have employed a modification of the method of measuring the phagocytic power of the blood, which was devised by Major W. B. Leishman, R.A.M.C.†

In the procedure described by this author equal volumes of a bacterial suspension of appropriate density and of blood drawn from the finger are measured off in a capillary tube, mixed on a slide and covered in with a cover-glass. The blood and bacterial culture are then left in contact for 15 minutes in an incubator standing at blood heat. After this interval the cover-glass is, if necessary, loosened from the slide by a drop of physiological salt solution, and the slide and cover-glass are drawn apart by a sliding movement.

* Wright and Windsor, 'Journal of Hygiene,' vol. 2, No. 4, Oct., 1902.

† 'Brit. Med. Journ.,' Jan. 11, 1902.

The films thus obtained are stained by Leishman's* modification of Romanovski's stain, and are subjected to examination under an immersion lens. By enumerating the bacteria ingested in a number of polynuclear white blood corpuscles and dividing, an average is obtained. This average is taken as the measure of the phagocytic power of the blood. It is compared, when comparative experiments are made, with the phagocytic power of a normal blood.

We have modified this method for our purposes (*a*) by conducting the phagocytosis in capillary tubes, making afterwards film preparations in the ordinary way; (*b*) by decalcifying the blood with citrate of soda, thus avoiding the complications introduced by blood coagulation, and making it possible to separate the white corpuscles from the blood fluids by centrifugalisation, decantation and washing.

Three different procedures, varying only in details, were employed in our experiments.

Procedure No. 1, *Employed where Nothing more than a Comparison between Bloods from Different Sources or Bloods Subjected to Different Conditions is Required.*

Having provided ourselves with a simple capillary pipette, furnished with a rubber teat and a pencil mark on the stem, we aspirate into the stem of the pipette—dividing off by bubbles of air in accordance with the procedure introduced by one of us—one volume of blood from the finger, one volume of a 1-per-cent. solution of citrate of soda in physiological salt solution, and one volume of a bacterial† suspension made by shaking up a 24-hour agar culture in physiological salt solution and centrifugalising so as to remove any bacterial clumps. We mix together the three equal volumes of blood, bacterial suspension and citrate of soda solution, by blowing these out upon a clean slide and re-aspirating several times in succession. Mixture completed, an aliquot portion of the mixed fluids, such as suffices for our purposes, is drawn up into the capillary stem, and the orifice of the capillary tube is sealed in the flame. This done, the pipette is placed either in an incubator standing at 37° C. or in a vessel of water kept at this temperature.

After the lapse of 15 minutes we break off the extremity of the pipette, carefully mix the contents so as to get an average sample. and proceed to make films, and then to stain them by Leishman's dye.

* 'Brit. Med. Journ.,' 1901.

† This bacterial suspension may conveniently contain about 10,000,000,000 bacteria in the cubic centimetre. The number may be readily adjusted by the help of the method of enumeration under the microscope described by one of us in the 'Lancet,' July 5, 1902.

Procedure No. 2, Employed where we Desire to Elicit Separately the Rôle *of the White Corpuscles and the Blood Fluids in Phagocytosis, and to Study the Effect Produced by Experimental Modifications of one or other of these Elements Separately.*

Having provided ourselves with a capsule with a recurved limb (such a capsule has already been figured in a previous communication),* we introduce into it such a quantity of mercury as will fill it to about one-third of its capacity. Having marked off by a pencil mark (made with a glass writing pencil) the level at which the upper surface of the mercury stands, we displace the mercury in such a manner as to cause it to occupy the middle instead of the lower region of the capsule. We again mark off on the outside of the capsule the upper limit of the mercury.

Then, emptying out this last, we bend round in the flame the curved limb in such a manner as to cause it to lie in the plane of the equator of the capsule. This enables us to siphon into it from a watch glass, filled and placed ready to hand, the citrate of soda solution. We introduce of this solution such a quantity as suffices to fill the capsule up to the level of the first pencil mark. This done, we draw blood from the finger and let it run into the capsule until the combined volume of citrate of soda solution and blood attains the level of the second pencil mark.

Having sealed up the upper orifice in the flame—rarefying as we do so the air in the interior of the capsule by the application of warmth—we shake up the contents and suspend the capsule by means of its curved limb into the receptacle of the hand centrifugal machine.

When centrifugalisation has caused the corpuscles to settle to the bottom, we pipette off and reserve the supernatant citrated plasma and replace it by physiological salt solution. In conducting this last operation we employ a capillary pipette, and we carry down its orifice to the very bottom of the capsule in such a manner as effectively to mix up the corpuscles and the newly added fluid. We wash and centrifugalise in this manner three times. The upper layers of the corpuscular deposit, containing as they do a large proportion of white corpuscles, supply the phagocytes required for experimentation.

In the experiments set forth below we mixed in each case three volumes of the upper layers of the washed corpuscular deposit, with three volumes of blood fluid, and one volume of a staphylococcus suspension, containing generally from 7000—10,000 million staphylococci per c.c. The mixture of corpuscles, blood fluid and staphylococci was kept in each case for 15 minutes at a temperature of 37° C., in order to give opportunity for the occurrence of phagocytosis.

* Wright, 'Roy. Soc. Proc.,' vol. 71, 1902.

Procedure No. 3, Employed where we desire to obtain Citrated Serum for comparison with the Citrated Plasma furnished by Procedure No. 2.

Where we desire to obtain citrated serum for comparison with the citrated plasma furnished by Procedure No. 2, we graduate our blood capsule in precisely the same manner. Having filled in with blood from the finger up to the first mark, we allow it to clot, and we then introduce into the capsule from a capillary pipette a sufficiency of citrate of soda solution to complete up to the second mark. Finally, we churn up the citrate of soda solution with the blood clot and then centrifugalise.

Accuracy of the Method and Special Points which come up for consideration in connection with it.

The accuracy of the method is attested by the concordant results set forth below of the large number of experiments which we conducted in duplicate. We desire to point out that the results incorporated below represent not exceptional fortunate achievements, but simply what may be obtained by the ordinary every-day application of the method.

Before dismissing the consideration of the experimental method, it may be well to elucidate very briefly three points which suggest themselves for consideration in connection with it.

The first of these relates to the calibre of the capillary tubes.

In our earlier experiments we considered it advisable, with a view to providing against a possible cause of fallacy, to conduct our experiments in capillary tubes of a standard calibre. The tubes were in each case calibrated by the method described by one of us,* to wit, by introducing into the wide end of a tube drawn out in the flame 5 c.mm. of mercury from an "automatic pipette," and marking off that portion of the capillary stem where this quantum of mercury formed a column 5 cm. in length. The experiments which we conducted with calibrated tubes are those which occupy the two next following sections of this paper.

In our later experiments, to wit, in the experiments which occupy the later sections of this paper, we discarded calibrated for uncalibrated tubes, making only the condition that the capillary tubes employed in comparative experiments should appear to the eye to be more or less comparable in calibre. It will be seen, on looking into our results, that the concordance obtained was not less in the case where uncalibrated tubes were employed than in the case where calibrated tubes were employed.

* 'Transactions of the Roy. Medico-Chirurg. Soc.,' vol. 86, and 'Lancet,' July 5, and Dec., 1902.

Different results, however, emerge when experiments in duplicate are conducted with tubes presenting extreme differences in calibre. In a series of comparative experiments, in which we employed in each case an almost hair-fine capillary tube as a fellow to a tube almost too large to be reckoned as a capillary tube, the results were irregular, being generally but not consistently in favour of the narrower tube.

A *second point* which comes up for consideration is the possible effect of the addition of citrate of soda to the blood.

The concentration of the solution in particular comes into consideration. Finding that phagocytosis is inhibited when the white corpuscles are bathed in a medium containing 3 per cent. of citrate of soda, we took the precaution to add to the blood in comparative experiments precisely the same amounts of citrate of soda. It may be noted that the morphological structure of the white corpuscles is extremely well preserved, and phagocytosis proceeds actively in a medium containing up to 1·5 per cent. of citrate of soda.

The *third* and last *point* to be considered relates to the maintenance of the activity of the phagocytes for a sufficient period after they have been withdrawn from the organism and have been subjected to the procedures described above. It will be manifest that, apart from a maintenance of the activity of the phagocytes under the conditions which come into consideration here, it would be impracticable to compare the results of experiments instituted in succession with one and the same quantum of washed corpuscles, or to compare the phagocytic power of different bloods unless in the case where these were withdrawn from the organism simultaneously.

A number of experiments undertaken with a view of obtaining information with regard to the point here raised have shown us that the phagocytic power is well maintained under the circumstances of our experiments. Even after the lapse of 3 days (our observations have not extended beyond this limit) the phagocytic power has not declined to less than one-half or one-third of that of the blood freshly drawn. We have found no indication of a variation within the space of a few hours.

These preliminary points having been dealt with, we may pass to the consideration of the problem to which attention was directed in the opening paragraph of this paper.

DOES THE SUBSTITUTION OF ANOTHER MEDIUM FOR THE (CITRATED) BLOOD PLASMA WHICH BATHES THE CORPUSCLES EXERT AN INFLUENCE ON PHAGOCYTOSIS ?

1. *Comparative Experiments with Citrated Plasma and Citrated Serum (obtained Respectively as Described in Connection with Procedures 2 and 3).*

EXPERIMENT 1.

A.—S. R. D.'s *plasma*, 3 vols.; staphylococcus suspension, 1 vol.; A. E. W.'s corpuscles, 3 vols.

Tube 1.—Phagocytic power (obtained by averaging the number of staphylococci ingested by 20 P.W.B.C.) ..... 34·6 } 34·1
Tube 2.— Do. do. do. 33·6 }

B.—S. R. D.'s *serum*, 3 vols.; staphylococcus suspension, 1 vol.; A. E. W.'s corpuscles, 3 vols.

Tube 1.—Phagocytic power (elicited as above) ... 35·6 } 34·7
Tube 2.— Do. do. ... 33·8 }

EXPERIMENT 2.

A.—A. E. W.'s *plasma*, 3 vols.; staphylococcus suspension, 1 vol.; A. E. W.'s corpuscles, 3 vols.

Tube 1.—Phagocytic power (elicited as above) ... 31·2 } 33·6
Tube 2.— Do. do. ... 36·0 }

B.—A. E. W.'s *serum*, 3 vols.; staphylococcus suspension, 1 vol.; A. E. W.'s corpuscles, 3 vols.

Tube 1.—Phagocytic power (elicited as above) ... 31·2 } 32·1
Tube 2.— Do. do. ... 33·0 }

It is clear that the phagocytic power is uninfluenced by the substitution of serum for plasma.

2. *Comparative Experiments with Ordinary (Uncitrated) Serum Unheated and Heated for* 10—15 *min. to* 60—65° *C. and then cooled.*

EXPERIMENT 1.

A.—A. E. W.'s *unheated serum*, 3 vols.; staphylococcus suspension, 1 vol.; A. E. W.'s corpuscles, 3 vols.

Tube 1.—Phagocytic power (bacteria in 20 P.W.B.C. enumerated and averaged) ..... 17·4
Tube 2.— Do. do. 19·8

B.—A. E. W.'s *heated serum*, 3 vols.; staphylococcus suspension, 1 vol.; A. E. W.'s corpuscles, 3 vols.

Tube 1.—Phagocytic power (bacteria in 52 P.W.B.C. enumerated and averaged) ..... 0·6
Tube 2.—Phagocytic power (bacteria in 46 P.W.B.C. enumerated and averaged)..... 3·4

EXPERIMENT 2.

A.—S. R. D.'s *unheated serum*, 3 vols.; staphylococcus suspension 1 vol.; S. R. D.'s corpuscles, 3 vols.

Tube 1.—Phagocytic power (bacteria in 20 P.W.B.C. enumerated and averaged) ..... 18·5
Tube 2.—Phagocytic power (bacteria in 22 P.W.B.C. enumerated and averaged) ..... 16·0

B.—S. R. D.'s *heated serum*, 3 vols.; staphylococcus suspension, 1 vol.; S. R. D.'s corpuscles, 3 vols.

Tube 1.—Phagocytic power (bacteria in 29 P.W.B.C. counted and averaged) ..... 1·5
Tube 2.—Phagocytic power (bacteria in 46 P.W.B.C. counted and averaged) ..... 1·8

EXPERIMENT 3.

A.—A. E. W.'s *unheated serum*, 3 vols.; staphylococcus suspension, 1 vol.; A. E. W.'s corpuscles, 3 vols.

Tube 1.—Phagocytic power (bacteria in 9 P.W.B.C. counted and averaged) ..... 25·4
Tube 2.—Phagocytic power (bacteria in 18 P.W.B.C. counted and averaged) ..... 16·0

B.—A. E. W.'s *heated serum*, 3 vols.; staphylococcus suspension, 1 vol.; A. E. W.'s corpuscles, 3 vols.

Tube 1.—Phagocytic power (bacteria in 20 P.W.B.C. counted and averaged) 0
Tube 2.— Do. do. do. 0

EXPERIMENT 4.

A.—S. R. D.'s *unheated serum*, staphylococcus suspension and corpuscles in the same proportions as before.

Phagocytic power (bacteria in 15 P.W.B.C. counted and averaged) ..... 15·7

B.—S. R. D.'s *heated serum*, staphylococcus supension, and corpuscles in the same proportions as before.

Phagocytic power (bacteria in 45 P.W.B.C. counted and averaged) ..... 0·2

These experiments show that we must ascribe an important *rôle* to the blood fluids in connection with phagocytosis.

For the alternative assumption, the supposition, to wit, that inhibiting elements are developed in the serum during the process of heating, is rebutted by the results of a series of control experiments, which showed that the phagocytes display no greater activity in a medium of physiological salt solution than in a medium of heated serum.

It is further rebutted by the circumstance that the activity of phagocytosis falls off at the same rate when the unheated serum is diluted with salt solution as when it is diluted with heated serum.

The experiment whose results are tabulated below illustrates this last point.

Results of a Comparison made between the Activating Power of (a) *Unheated Serum diluted in Heated Serum, and* (b) *Unheated Serum diluted in Physiological Salt Solution.*

In each case 3 vols. of serum dilution were mixed with 1 vol. of staphylococcus suspension and 3 vols. of washed corpuscles.

Dilution in which the unheated serum was employed.	Average phagocytic power of the P.W.B.C. in the case where the unheated serum was diluted with previously heated serum.	Average phagocytic power of the P.W.B.C. in the case where the unheated serum was diluted with physiological salt solution.
3-fold	..	34·2
6-fold	27·4	27·2
12-fold	23·1	30·5
24-fold	20·6	24·8
48-fold	5·0	4·95
96-fold	..	0·8
192-fold	..	0·6

It is clear that we may conclude that the heated serum, like the salt solution, acts merely as an inert diluent, and that we may, in referring to such heated serum, characterise it simply as "inactivated serum." It is further clear that we may legitimately ascribe the small amount of phagocytosis which occurred in Experiments 1, 2, and 4 *supra*, to the presence of a residuum of unheated serum, which the washing operations had failed to separate from the corpuscles.

DO THE BLOOD FLUIDS CO-OPERATE IN PHAGOCYTOSIS BY EXERTING A DIRECT "STIMULATING" EFFECT UPON THE PHAGOCYTES, OR BY EFFECTING A MODIFICATION IN THE BACTERIA?

The following experiments were instituted with a view to elucidating the problem as to the nature of the activating influence exercised by the blood fluids. It will be seen that a comparison is in each case instituted between serum inactivated (by heating) before it came in contact with either bacteria or white corpuscles, and serum inactivated after it had come in contact with the bacteria, but before it had come in contact with the white corpuscles:—

EXPERIMENT 1.

A.—S. R. D.'s *inactivated* serum, 3 vols.; staphylococcus suspension (previously heated to 60° C. for 15 minutes and cooled), 1 vol.; S. R. D.'s corpuscles, 3 vols.

Tube 1.—Phagocytic power (bacteria in 20 P.W.B.C. counted and averaged) ..... 3·4

Tube 2.— Do. do. 3·35

B.—S. R. D.'s *unheated* serum, 3 vols.; digested at 37° C. for 15 minutes, with 1 vol. of staphylococcus suspension, then heated to 60° C. for 15 minutes and cooled.

4 vols. of the above mixed with 3 vols. of S. R. D.'s corpuscles.

Tube 1.—Phagocytic power (bacteria in 20 P.W.B.C. counted and averaged) ..... 27·5

Tube 2.— Do. do. 28·9

EXPERIMENT 2.

A.—A. E. W.'s *inactivated* serum, 3 vols.; staphylococcus suspension, 1 vol.; digested together for 15 minutes at 37° C., then heated for 10 minutes to 60° C. and cooled.

4 vols. of the above mixed with 3 vols. of S. R. D.'s corpuscles.

Tube 1.—Phagocytic power (bacteria in 20 P.W.B.C. counted and averaged) ..... 4·0

Tube 2.— Do. do. 3·2

B.—A. E. W.'s *unheated* serum, 3 vols.; staphylococcus suspension, 1 vol.; digested together for 15 minutes at 37° C., then heated for 10 minutes to 60° C. and cooled.

4 vols. of the above added to 3 vols. of S. R. D.'s corpuscles.

Tube 1.—Phagocytic power (bacteria in 24 P.W.B.C. counted and averaged) 33

Tube 2.—Phagocytic power (bacteria in 19 P.W.B.C. counted and averaged) 36

EXPERIMENT 3.

A.—S. R. D.'s *inactivated* serum, 3 vols.; staphylococcus suspension (previously heated to 75° C. and cooled), 1 vol.; digested together for 15 minutes at 37° C.

4 vols. of the above added to 3 vols. of S. R. D.'s corpuscles.

Phagocytic power (bacteria in 30 P.W.B.C. counted and averaged) ..... 4·2

B.—S. R. D.'s *unheated* serum, 3 vols.; staphylococcus suspension (previously heated to 75° C. and cooled), 1 vol.; digested together for 15 minutes at 37° C., then heated for 10 minutes to 60° C., and cooled.

4 vols. of the above added to 3 vols. of corpuscles.

Tube 1.—Phagocytic power (bacteria in 15 P.W.B.C. counted and averaged) ..... 28·2

Tube 2.— Do. do. 31·0

We have here conclusive proof that the blood fluids modify the bacteria in a manner which renders them a ready prey to the phagocytes.

We may speak of this as an "*opsonic*" effect (opsono—*I cater for; I prepare victuals for*), and we may employ the term "opsonins" to designate the elements in the blood fluids which produce this effect.

DOES THE UNHEATED SERUM CONTAIN, IN ADDITION TO ELEMENTS WHICH RENDER THE BACTERIA MORE LIABLE TO PHAGOCYTOSIS (OPSONINS), ALSO ELEMENTS WHICH DIRECTLY STIMULATE THE PHAGOCYTES (STIMULINS)?

We have sought to elucidate this question by three separate methods.

In a first series of experiments, we experimented with staphylococci which had been exposed to high temperatures (115° C.) with the design of rendering them insusceptible to the opsonic power of the blood fluids. Our expectations from this method—expectations based on the fact that we had noticed that typhoid bacilli acquired, when heated to over 70° C., a resistance to the bacteriolytic effect of the blood fluids—were unrealised. We found that the quantitative differences between the phagocytosis in heated and unheated serum respectively were not less in the case of staphylococci which had been exposed to a temperature of 115° C., than in the case of staphylococci which had not been subjected to high temperatures.

In a second series of experiments we substituted for suspensions of staphylococci suspensions of particles, which we assumed would be uninfluenced by the opsonic power of the blood. The results of these

experiments, conducted both with carmine particles and with Indian ink, were inconclusive by reason of the circumstance that we were not able to obtain any satisfactory enumerations. An impression was, however, left on our minds that phagocytosis was in every case more active in unheated than in the heated serum.

A third method of experimentation was then resorted to. In a first operation we mixed and digested together at blood heat a suspension of staphylococci and unheated serum. After allowing what we supposed would be a sufficient interval for the exhaustion of the effect of the serum upon the bacteria, we divided the mixture into two portions. While the first of these portions was mixed with the corpuscles without undergoing any further treatment, the other was heated to 60° C., and cooled before it was so mixed. In each case the phagocytic power exerted was greater in the case where the heating was omitted, and the differences were not less marked where the serum had been digested with the bacteria for 50 minutes and 1 hour respectively than in the case where it had been digested with these only for 15 minutes.

These results are ambiguous.

The question as to whether the blood fluids contain, in addition to opsonins, also an element which directly stimulates the phagocytes, remains for the present unsolved.

The third series of experiments, which has just been adverted to, is subjoined :—

EXPERIMENT 1.

S. R. D.'s serum, 3 vols. ; digested with staphylococcus suspension, 1 vol., for 15 minutes at 37° C.

A.—4 vols. of the above mixture heated to 60° C. for 15 minutes, then cooled and added to 3 vols. of S. R. D.'s corpuscles.

Tube 1.—Phagocytic power (bacteria in 16 P.W.B.C. enumerated and averaged) ..... 22·0

Tube 2.—Phagocytic power (bacteria in 31 P.W.B.C. enumerated and averaged) ..... 20·7

B.—4 vols. of the above mixture added directly to 3 vols. of S. R. D.'s corpuscles.

Tube 1.—Phagocytic power (bacteria in 16 P.W.B.C. counted and averaged) 27

Tube 2.—Phagocytic power (bacteria in 28 P.W.B.C. counted and averaged) 28

EXPERIMENT 2.

S. R. D.'s unheated serum, 3 vols. ; digested with staphylococcus suspension, 1 vol., for 50 minutes at 37° C.

A.—4 vols. of the above mixture heated to 60° C. for 20 minutes, then cooled and added to 3 vols. of S. R. D.'s corpuscles.

Tube 1.—Phagocytic power (bacteria in 20 P.W.B.C. enumerated and averaged) ..... 13·5
Tube 2.— Do. do. 17·1

B.—4 vols. of the above mixture added directly to 3 vols. S. R. D.'s corpuscles.

Tube 1.—(Bacteria in 15 P.W.B.C. enumerated and averaged) .... 40·6
Tube 2.— Do. do. 44·5

EXPERIMENT 3.

S. R. D.'s unheated serum, 3 vols.; 1 vol. of staphylococcus suspension; digested together 1 hour at 37° C.

A.—4 vols. of the above mixture heated to 60° C. for 10 minutes, cooled and added to 3 vols. of S. R. D.'s corpuscles.

Tube 1.—Phagocytic power (bacteria in 16 P.W.B.C. enumerated and averaged) ..... 15·1

B.—4 vols. of the above mixture added directly to 3 vols. of S. R. D.'s corpuscles.

Tube 1.—Phagocytic power (bacteria in 16 P.W.B.C. counted and averaged) ..... 22·1

In conclusion we would briefly refer to the following points:—

The opsonic power of the blood fluids disappears gradually on standing, even when the serum is kept in a sealed capsule sheltered from the light.

After 5 or 6 days we have found the opsonic power of the serum kept under these conditions to stand at little more than half of what it was originally.

The opsonic power of the blood fluids is but little impaired by the action of heat until these have been exposed to temperatures above 50° C. The following are the results of a typical experiment:—Phagocytic power obtained with the serum before exposure to heat, 12·7; with the same serum heated for 10 minutes to 45° C., 13·1; with the same serum heated for 10 minutes to 50° C., 10·2; with the same serum heated for 10 minutes to 55° C., 5·7.

The opsonic power of the serum is diminished when this last has been digested with typhoid bacteria. This "anti-opsonic" effect may compared with the "anti-bactericidal" effect* obtained on digesting the serum with typhoid or cholera cultures.

* Wright and Windsor, 'Journal of Hygiene,' vol. 2, No. 4, Oct., 1902.

The opsonic power of the blood fluids is diminished while the phagocytic capacity of the W.B.C. is preserved when the blood fluids and corpuscles are separately digested with Daboia venom. In the anti-opsonic effect, exerted by the venom on the blood fluids, we have probably the explanation of the reduced resistance to septic invasion which supervenes upon viper bites.

It would seem probable that the bacteriolytic, bactericidal, and bacterio-opsonic effects exerted by the blood fluids are each in their degree manifestations of a digestive power exerted by the blood fluids on bacteria brought into contact with them.

Lastly, a fact which has a practical importance in connection with the study of immunity may be adverted to. It will be manifest that we have not exhausted the study of a condition of immunity when we have measured the phagocytic power of the white corpuscles, and the agglutinating, bacteriolytic, and bactericidal powers of the blood fluids. We must, in connection with these last, take into consideration also the opsonic effect.

A concrete example may be added to show the kind of elucidation which may be gained from an inquiry which takes into consideration also the factor last mentioned.

The condition of immunity to staphylococcus which can be induced in patients unduly susceptible to staphylococcus infections, by the inoculation of properly adjusted doses of a sterilised staphylococcus culture is, as was shown by one of us, associated with an increase of the phagocytic power* of the white blood corpuscles, and is unaccompanied by any development of a bactericidal power in the blood fluids.

The result of the subjoined blood examinations undertaken upon a patient who had been subjected to two successive therapeutic inoculations of a sterilised staphylococcus culture, suggests that the increased phagocytic power may depend upon an increase in the opsonic power of the blood fluids.

A.—A. E. W.'s serum, 3 vols.; staphylococcus suspension, 1 vol.; and A. E. W.'s washed corpuscles, 3 vols.

Tube 1.—Phagocytic power (bacteria in 20 P.W.B.C. counted and averaged) ..... 17·4

Tube 2.—Phagocytic power (bacteria in 26 P.W.B.C. counted and averaged) ..... 19·9

B.—The patient's serum, 3 vols.: staphylococcus suspension, 1 vol.; the patient's washed corpuscles, 3 vols.

Tube 1.—Phagocytic power (bacteria in 15 P.W.B.C. counted and averaged) 35

Tube 2.— Do. do. 36

* 'Lancet,' March 29, 1902.

C.—The patient's serum, 3 vols.; staphylococcus suspension, 1 vol.; A. E. W.'s washed corpuscles, 3 vols.

Tube 1.—	Phagocytic power (bacteria in 15 counted and averaged)	P.W.B.C. 30
Tube 2.—	Do. do.	26

"The Magnetic Expansion of some of the less Magnetic Metals." By P. E. Shaw, B.A., D.Sc. Communicated by Professor J. H. Poynting, F.R.S. Received May 22,—Read June 18, 1903.

1. Abundant research has been made on the magnetic expansion of iron, nickel, and cobalt, notably as regards the exact relation between field (H) and expansion per unit length ($\delta l/l$), by S. Bidwell* and H. Nagaoka.† Bismuth also has been investigated by Bidwell, C. G. Knott, Van Aubel, and A. P. Wills. But there seems to be no recorded research on any materials other than the four mentioned.

Outside the ferro-magnetic group bismuth has the largest susceptibility (k) of any substance; and the tacit assumption seems to have been made that if bismuth shows no change in length as the field varies, it is vain to look for it in less susceptible metals.

But in the case of the ferro-magnetics there is no direct relation between k and $\delta l/l$. Thus, iron has maximum susceptibility six times as much as nickel, and yet expands far less for any known field.

Again, cobalt has maximum susceptibility one-eighth of that of iron, yet expands about as much.

There being, therefore, no close relation between susceptibility and magnetic expansion, it seems possible that there may be appreciable movement for large fields in the case of metals outside the ferro-magnetic group. This paper gives an account of tests applied to bismuth, silver, aluminium, copper, zinc, brass, bronze, lead, and tin. (Not much importance should be attached to the results for lead and tin owing to the softness of these metals; they tend to work loose in their fittings at each end.) The work has taken from first to last nearly two years: the specimens have been repeatedly changed and the magnetic and measuring parts of the apparatus modified in various ways. In this way searching tests have been applied to the investigation.

For a long time it appeared (1) that all these metals contracted, the contraction being roughly proportional to the field, (2) that all the metals showed permanent magnetisation, on the hysteresis principle,

* 'Phil. Trans.,' A, 1888.
† 'Phil. Mag.,' Jan., 1894.